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L3	L2 and (phosphrothioate or phosphoramidate$1)	25	L3
L2	L1 and (end$1 or termin$3)	113	L2
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L6: Entry 20 of 21 File: USPT Jun 9, 1998

DOCUMENT-IDENTIFIER: US 5763594 A
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TITLE: 3' protected nucleotides for enzyme catalyzed template-independent creation of phosphodiester bonds

Abstract Text (1):
A method for the stepwise creation of phosphodiester bonds between desired nucleosides resulting in the synthesis of polynucleotides having a predetermined nucleotide sequence by preparing an initiation substrate containing a free and unmodified 3'-hydroxyl group; attaching a mononucleotide selected according to the order of the predetermined nucleotide sequence to the 3'-hydroxyl of the initiating substrate in a solution containing a catalytic amount of an enzyme capable of catalyzing the 5' to 3' phosphodiester linkage of the 5'-phosphate of the mononucleotide to the 3'-hydroxyl of the initiating substrate, wherein the mononucleotide contains a protected 3'-hydroxyl group, whereby the protected mononucleotide is covalently linked to the initiating substrate and further additions are hindered by the 3'-hydroxyl protecting group. Methods in which a mononucleotide immobilized on a solid support is added to a free polynucleotide chain are also disclosed.

Brief Summary Text (5):
The phosphoramidite method of phosphodiester bond formation and oligonucleotide synthesis represents the current state of the art employed by most laboratories for the coupling of desired nucleotides without the use of a template. In this method, the coupling reaction is initiated by a nucleoside attached to a solid support. The 5'-hydroxyl group of the immobilized nucleoside is free for coupling with the second nucleoside of the chain to be assembled. Since the growing oligonucleotide chain projects a 5'-hydroxyl available for reaction with a mononucleotide, the direction of synthesis if referred to as 3' to 5'.

Brief Summary Text (6):
Each successive mononucleotide to be added to the growing oligonucleotide chain contains a 3'-phosphoramidate moiety which reacts with the 5'-hydroxyl group of the support-bound nucleotide to form a 5' to 3' internucleotide phosphodiester bond. The 5'-hydroxyl group of the incoming mononucleotide is protected, usually by a trityl group, in order to prevent the uncontrolled polymerization of the nucleosides. After each incoming nucleoside is added, the protected 5'-hydroxyl group is deprotected, so that it is available for reaction with the next incoming nucleoside having a 3'-phosphoramidite group and a protected 5'-hydroxyl. This is followed by deprotection and addition of the next incoming nucleotide, and so forth.

Brief Summary Text (14):
There are many enzymes capable of catalyzing the formation of phosphodiester bonds. One class of such enzymes, the polymerases, are largely template dependent in that they add a complementary nucleotide to the 3' hydroxyl of the growing strand of a double stranded polynucleotide. However, some polymerases are template independent and primarily catalyze the formation of single stranded nucleotide polymers. Another class of enzyme, the ligases, are template independent and form a phosphodiester bond between two polynucleotides or between a polynucleotide and a mononucleotide.

Brief Summary Text (15):
Addition of single nucleotides to DNA fragments, catalyzed by deoxynucleotidyl terminal transferase (TdTase), has previously been described by Deng and Wu, Meth. Enzymol., 100:96-116, 1983. These reaction conditions did not involve transient protection of the 3'-hydroxyl nor were they intended to be used for the sequential creation of phosphodiester bonds to synthesize a predetermined nucleotide sequence. The presence of unprotected 3'-hydroxyls resulted in a highly heterogeneous population

of reaction products.

Brief Summary Text (18):
These prior attempts at synthesizing oligonucleotides using a template independent polymerase were extremely inefficient resulting in the production of very short oligonucleotides. The inefficiency of these methods made these methods useless for practical synthesis of oligonucleotides.

Brief Summary Text (19):
The present invention allows the creation of phosphodiester bonds between nucleotides using a template independent polymerase to create oligonucleotides having a predetermined sequence. This enzyme catalysis can vastly improve the efficiency of phosphodiester bond formation between desired nucleotides compared to current techniques of chemical coupling and can be carried out in the presence of other biological molecules such as pre-existing sequences of single or double stranded DNA as well as other types of enzymes. In addition, the very high specificity inherent to enzyme catalysis allows only coupling of a 5'-phosphate to a 3'-hydroxyl. The coupling of two mononucleosides, as well as various other side reactions inherent to chemical coupling techniques, simply do not occur.

Brief Summary Text (23):
A number of methods have been discovered by which the 3'-hydroxyl group of a deoxynucleotide triphosphate can be effectively protected and deprotected and wherein the protected nucleotide is utilized by a template independent polymerase to create a phosphodiester bond permitting the synthesis of oligonucleotides or polynucleotides having a desired predetermined sequence.

Brief Summary Text (29):
In other embodiments, steps (b) and (c) are repeated at least once to add additional nucleotides to the initiating substrate by alternatively adding a nucleoside 5'-triphosphate with a removable blocking moiety at its 3' position, deblocking the 3' position of the terminal nucleoside and then adding another nucleoside 5'-triphosphate with a removable blocking group at its 3' position. Repetition of steps (b) and (c) can also be carried out to produce an oligonucleotide or polynucleotide having a predetermined sequence.

Brief Summary Text (31):
The present invention contemplates embodiments in which the substrate is immobilized on a solid support. Preferred solid supports include cellulose, Sepharose, controlled-pore glass, silica, Fractosil, polystyrene, styrene divinyl benzene, agarose, and crosslinked agarose and the like.

Brief Summary Text (32):
The present invention contemplates the use of template independent polynucleotide polymerases such as terminal deoxynucleotidyl transferase from any number of sources including eukaryotes and protharyotes.

Brief Summary Text (33):
The methods of the present invention utilize removable blocking moieties that block the 3' position of nucleoside 5'-triphosphates used in the methods. Preferred removable blocking moieties can be removed in under 10 minutes to produce a hydroxyl group at the 3' position of the 3' nucleoside. Removable blocking groups contemplated include carbonitriles, phosphates, carbonates, carbamates, esters, ethers, borates, nitrates, sugars, phosphoramidates, phenylsulfenates, sulfates and sulfones.

Brief Summary Text (35):
The present invention also contemplates methods in which the nucleoside 5'-triphosphate having the removable blocking moiety at its 3' position is immobilized in a solid support and reacted with free initiating substrates. The solid support is linked to the nucleoside 5'-triphosphate at the 3'-hydroxyl group, thereby acting as a removable blocking moiety at the 3' position. Attachment of the nucleoside to the support is transient, thereby enabling the release of the newly synthesized product from the support and regeneration of the free and unmodified 3'-hydroxyl to allow the next nucleotide addition to occur.

Brief Summary Text (36):
Thus, in some embodiments of the present invention the deblocking solution would remove the removable blocking moiety at the position of the nucleoside and thus release the growing polynucleotide from the solid support.

Drawing Description Text (2):
FIG. 1. A diagram showing enzymatic synthesis of an oligonucleotide using a template independent polymerase and a nucleoside 5' triphosphate having a removable blocking moiety at its 3' position is shown.

Detailed Description Text (14):
Promoter: A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

Detailed Description Text (15):
Oligonucleotide: A chain of nucleosides which are linked by internucleoside linkages which is generally from about 2 to about 50 nucleosides in length. They may be chemically synthesized from nucleoside monomers or produced by enzymatic means. The term oligonucleotide refers to a chain of nucleosides which have internucleosidyl linkages linking the nucleoside monomer and, thus, includes oligonucleotide containing nucleoside analogs, oligonucleotide having internucleosidyl linkages such that one or more of the phosphorous group linkages between monomeric units has been replaced by a non-phosphorous linkage such as a formacetal linkage, a thioformacetal linkage, a sulfamate linkage, or a carbamate linkage. It also includes nucleoside/non-nucleoside polymers wherein both the sugar and the phosphorous moiety have been replaced or modified such as mopholino base analogs, or polyamide base analogs. It also includes nucleoside/non-nucleoside polymers wherein the vase, the sugar, and the phosphate backbone of a nucleoside are either replaced by a non-nucleoside moiety or wherein a non-nucleoside moiety is inserted into the nucleoside/non-nucleoside polymer. Thus an oligonucleotide may be partially or entirely phophonothioates, phosphorothioate phosphorodithioate phosphoramidate or neutral phosphate ester such as phosphotriesters oligonucleotide analogs.

Detailed Description Text (18):
Generally, the present invention provides methods for synthesizing oligonucleotides and polynucleotides having a predetermined sequence using a template independent polymerase and nucleoside having the 3' position blocked with a removable blocking moiety so that single nucleosides are added to the growing oligonucleotide. Single nucleosides are added to the growing chain by removing the blocking moiety at the 3' position of the terminal nucleoside of the growing oligonucleotide so that the next blocked nucleoside can be added to the oligonucleotide. This process is then repeated until the oligonucleotide having the predetermined sequence is produced.

Detailed Description Text (24):
This additional step regenerates a reactive atom at the 3' position of the terminal nucleoside so that this atom can be used to form a bond with the next nucleoside and thus extend the length of the oligonucleotide by one nucleoside.

Detailed Description Text (29):
The initiating substrates of the present invention include the termini of polynucleotides frequently generated and used in various cloning and molecular biology techniques. Examples of these initiating substrates include the termini of DNA or RNA vectors, single-stranded or double-stranded fragments, single-stranded or double-stranded RNA fragments and RNA or DNA oligonucleotides.

Detailed Description Text (30):
In the preferred embodiments, initiating substrates will consist wholly or in part of an oligo- or polynucleotide. The initiating substrate can be any arrangement of nucleosides which enables the enzyme to create a phosphodiester bond between the 3'-hydroxyl of a nucleoside and the 5'-phosphate of a mononucleotide. Initiating substrates may be based wholly or in part on ribonucleic acids (RNA) or deoxyribonucleic acids (DNA) and may be single stranded or multi-stranded. In addition, initiating substrates can include other types of naturally occurring or synthetic molecules (non-nucleosides) which may enable or enhance the ability of the

enzyme to create a phosphodiester bond or which may facilitate the manipulation of reaction components and by-products. An example of this would be a linker molecule (commonly used linkers consist of C, O, N, and H e.g. Affi-Gel.TM. 10: R--OCH.sub.2 CONH(CH.sub.2).sub.2 NHCO(CH.sub.2).sub.2 COON(CH.sub.2).sub.2 which would serve to provide a convenient method for attaching an initiating substrate to a solid support.

Detailed Description Text (31):
The sequential creation of phosphodiester bonds and hence the addition of nucleotides to the initiating substrate may be performed entirely in solution, or the initiating substrate may be attached to an insoluble matrix. Attachment to an insoluble matrix will permit the rapid separation of the substrate from unreacted reagents in order to prepare the substrate for the addition of the next nucleotide. For this reason, the substrate is preferably affixed to a solid support matrix during each reaction creating a phosphodiester bond.

Detailed Description Text (32):
Insoluble matrices suitable for use as solid supports include cellulose, Sepharose.TM., controlled-pore glass (CPG), polystyrene, silica, agarose, and the like.

Detailed Description Text (33):
Reagents, buffers and solvents suitable for use with the present invention are capable of flowing through the solid support matrix, by which means the initiating substrate is brought into contact with these materials. The growing nucleotide chain remains attached to the solid support as the various reagents, buffers and solvents sequentially flow therethrough. The solid support matrix is preferably contained within a synthesis column, to which reagents, buffers and solvents are provided.

Detailed Description Text (34):
Attachment of the initiating substrate to the solid support can be by covalent bonding. Numerous methods for the covalent attachment of molecules to insoluble matrices have been described and are well understood by those of ordinary skill in the art. In the preferred embodiment an oligonucleotide chain may be linked to alkylamine derivatized polystyrene or CPG by way of a covalent phosphoramidate bond although numerous strategies for linking oligonucleotides to solid supports have been described. The choice of an appropriate linking strategy will depend on the specific requirements of stability, charge interactions, solubility and the like.

Detailed Description Text (35):
Alternatively, attachment of the initiating substrate to the solid support can be by non-covalent interactions. Numerous methods for the transient attachment of molecules to insoluble matrices have been described and are well understood by those of ordinary skill in the art. For example, an oligonucleotide derivative containing single or multiple biotin molecules may be attached to avidin-agarose or streptavidin-agarose to form a non-covalent linkage between the oligonucleotide and the insoluble agarose matrix.

Detailed Description Text (36):
In general, it is envisioned that single and double stranded oligo- and polynucleotides based on DNA or RNA may be covalently or non-covalently bound to solid supports to form a variety of initiating substrates. Regardless of the strategy employed to attach an initiating substrate to an insoluble matrix, a nucleoside with a free and unmodified 3'-hydroxyl group will always be available for enzyme catalyzed creation of a phosphodiester bond.

Detailed Description Text (38):
Mononucleotides are added to the free and unmodified 3'-hydroxyl group of the initiating substrate by reacting the substrate with the 5'-phosphate of the selected mononucleotide in the presence of a catalytic amount of an enzyme capable of creating the phosphodiester bond covalently linking the 5'-phosphate of the mononucleotide with the 3'-hydroxyl of the substrate. The enzyme is preferable a template independent enzyme such as a template independent polynucleotide polymerase. Template independent enzymes such as template independent polynucleotide polymerases are capable of catalyzing the formation of a phosphodiester bond between the nucleotides without requiring the presence of a complementary nucleotide strand for activity. Thus, the

template independent enzymes such as template independent polynucleotide polymerases are able to catalyze the formation of single-stranded nucleic acid polymers without requiring a complementary nucleic acid strand to act as a template. Examples of template independent polynucleotide polymerases include terminal deoxynucleotidyl transferases. Template independent polynucleotide polymerases can be isolated from a number of sources including calf thymus and other sources of lymphocytes. A particularly preferred polymerase is terminal deoxynucleotidyl transferase (TdTase, EC 2.7.7.31).

Detailed Description Text (55):
Additional well known removable blocking moieties useful for protecting for hydroxyls include carbonitriles, phosphates, carbonates, carbamates, borates, nitrates, phosphoramidates, and phenylsulfenates. Most of these chemical modifications to the nucleotide can be removed by chemical reactions. Some modifications may also be removed by enzymatic digestion resulting in the regeneration of the 3' hydroxyl. These would include phosphates, glycosides, and certain esters.

Detailed Description Text (56):
In other embodiments, a nucleoside 5'-phosphate of the present invention has a removable blocking moiety protecting the 3' position having the following formula: ##STR12## wherein R2 is triphosphate, diphosphate or monophosphate; and wherein R1 is selected from the group consisting of phosphate, phosphoramidate and phosphoramide. In preferred embodiments the nucleoside 5' phosphate of the above formula has an R2 group which is triphosphate and an R1 group which is phosphate.

Detailed Description Text (61):
Attachment of the nucleotide having a removable blocking moiety protecting the 3'-position to the free and unmodified 3'-hydroxyl of the initiating substrate is then accomplished by reacting [incubating] the aforementioned nucleotide and the substrate with an enzyme capable of forming a phosphodiester bond between the two. Specifically, this bond would link the 5'-phosphate of the mononucleotide with the 3'-hydroxyl of the initiating substrate. This reaction can be performed either free in solution or, in one embodiment of the invention, the initiating substrate is immobilized on a solid support.

Detailed Description Text (62):
Particularly preferred are removable blocking moieties and deblocking reaction conditions that allow the blocking moiety to be removed in under 10 minutes to produce a hydroxyl group at the 3' position of the 3'-terminal nucleoside. Other preferred removable blocking moieties and deblocking conditions allow the blocking moiety to be removed in less than 8, 7, 6, 5, 4, 3, 2, or 1 minutes,

Detailed Description Text (70):
While the substrate containing a free and unmodified 3'-hydroxyl group and the mononucleotide having the removable blocking moiety protecting the 3'-hydroxyl group can be reacted in the presence of the TdTase in the buffer solution, the substrate is preferably immobilized on a solid support, and more preferably in a synthesis column to which the buffer solution containing the reaction components is delivered.

Detailed Description Text (71):
After the appropriate incubation time, the enzyme, unreacted mononucleotide, buffer and divalent cation are separated from the initiating substrate. If the reaction was performed using a free and soluble substrate, it can be separated by conventional size exclusion chromatography or similar types of separation techniques including but not limited to ion exchange chromatography and affinity chromatography. For initiating substrates immobilized on solid supports, separation is achieved by washing the support with water or a suitable buffer.

Detailed Description Text (73):
After the appropriate incubation time, capping reagents are separated from the initiating substrate. If the reaction was performed using a soluble substrate, it can be separated by conventional size exclusion chromatography or similar types of separation techniques including but not limited to ion exchange and affinity chromatography. For initiating substrates immobilized on solid supports, separation is achieved by washing the support with water or a suitable buffer.

Detailed Description Text (77):
If the removal or deblocking reaction is performed in solution, the deprotection reagents are simply added to the solution. If the reaction is performed with the initiating substrate immobilized on a solid support, then the hydroxyl group regeneration step is performed by washing the solid support with the deprotection reagents. When synthesis columns are utilized to contain the solid support, the hydroxyl group regeneration step is performed by washing the column with the appropriate agents.

Detailed Description Text (78):
After the appropriate period for removal, the initiating substrate (including both those that received an additional nucleotide and those that are capped) is again separated from the other reaction components. If the reaction was performed using a soluble substrate, it can be separated by conventional size exclusion chromatography or similar types of separation techniques including but not limited to ion exchange and affinity chromatography. For initiating substrates immobilized on solid supports, separation is achieved by washing the support with water or a suitable buffer.

Detailed Description Text (80):
Cleavage of a newly synthesized polynucleotide strand from the solid support and/or from the initiating substrate can be accomplished by either chemical or enzymatic reactions. In the case of a chemical reaction, if the initiating substrate terminal nucleoside (containing the free and unmodified 3'-hydroxyl group) is a deoxyguanosine methylated at the 7 position of the base:

Detailed Description Text (84):
The combined initiating substrate and object polynucleotide can be cleaved from the solid support by chemical methods. How the cleavage is performed will depend upon the nature of the initiating substrate and how it was attached to the solid support. Covalent labile bonds, such as for example a trityl group, can be cleaved by washing the support with an appropriate protic acid. Numerous other cleavage strategies have been described. In the case of a non-covalent attachment, as for example avidin-biotin binding, release of the combined substrate and object polynucleotide will occur upon incubation with 8M guanidine-HCl, pH 1.5.

Detailed Description Text (85):
If the entire synthesis was performed using a soluble initiating substrate, the initiating substrate containing the object polynucleotide can be separated from the various capped oligo- and polynucleotides by conventional chromatographic techniques, such as polyacrylamide gel electrophoresis. Similarly, if the initiating substrate is cleaved from the object polynucleotide by chemical or enzymatic means (e.g. by reaction with piperidine or by restriction endonucleases digestion as described above) conventional chromatography can be used to purify the object polynucleotide.

Detailed Description Text (86):
If the synthesis was performed using an initiating substrate immobilized to a solid support, cleavage from the solid support can be accomplished by either chemical or enzymatic means to retrieve either the combined initiating substrate and object polynucleotide or the object polynucleotide alone. In each instance, the object polynucleotide will be contaminated with capped oligo- and polynucleotides which can be separated from the object polynucleotide by polyacrylamide gel electrophoresis.

Detailed Description Text (87):
An alternative strategy for the synthesis and recovery of the object polynucleotide involves immobilization of the nucleotide. In this instance, the nucleotide is protected at the 3'-hydroxyl by a linker which is attached to a solid support. The linker attachment to the nucleotide can be by an ester or by any of the aforementioned protecting group strategies. Solid supports containing various functional groups (e.g. amines, amides, biotin, avidin, and the like) are generally available and can be adapted to the particular requirements of the nucleotide linker. For example, a nucleotide linker containing a biotin molecule can be bound to agarose using an avidin functional group attached to the agarose.

Detailed Description Text (88):

Using an immobilized nucleotide, the TdTase reaction would join a free initiating substrate, in solution, to the immobilized nucleotide, thereby immobilizing only those initiating substrates which have participated in the enzyme reaction. Initiating substrates which had not participated in the TdTase reaction would be easily removed by rinsing the solid support with an appropriate buffer. Regeneration of the 3' hydroxyl on the initiating substrate is accomplished by the same techniques as described previously.

Detailed Description Text (89):
Subsequent to the regeneration and cleavage step, the initiating substrate is rinsed away from the solid support and separated from the regeneration/cleavage solution containing free nucleotides by conventional techniques such as size exclusion chromatography, ion exchange or affinity chromatography. The next immobilized nucleotide, contained on a new population of solid support particles, is then mixed with the initiating substrate and the appropriate buffers in order to repeat the TdTase coupling reaction.

Detailed Description Text (90):
By immobilizing the nucleotide rather than the initiating substrate, a capping reaction is obviated since the object polynucleotide is separated from unreacted initiating substrate at every cycle. Similarly, if the cleavage reaction fails to release all of the object polynucleotide chains, those polynucleotides which continue to be attached to the solid support are removed prior to the subsequent TdTase reaction.

Detailed Description Text (91):
It is envisioned that various newly synthesized polynucleotide chains will subsequently be joined together by a polymerase/ligase type of reaction in order to form longer polynucleotide sequences that are in a double stranded form. For example, newly synthesized polynucleotides A and B may have the structures depicted below: ##STR14## where p(dN) is the predetermined object polynucleotide sequence unique to either the A or B polynucleotide. In the presence of the Klenow fragment of DNA polymerase I, and T4 DNA ligase, as well as the appropriate buffers and nucleotides, a double stranded polynucleotide will be formed in which the two object polynucleotides have been "stitched" together to form the longer double stranded polynucleotide C: ##STR15##

Detailed Description Text (92):
This reaction can be performed when one of the polynucleotides is still attached to a solid support or when both polynucleotides have been released into solution by the techniques described previously.

Detailed Description Text (102):
The compositions contemplated by the present invention includes compositions in which the template independent polynucleotide enzyme present is a template independent polynucleotide polymerase. Examples of preferred template independent polynucleotide polymerases include TdTase and enzymes with similar activities.

Detailed Description Text (106):
The present invention contemplates the incorporation of the method described herein in an automated process in an apparatus and in devices. For example, the various buffer and reagent solutions of the inventive process can be provided to synthesis columns containing initiating substrates affixed to solid support matrices by the use of flexible tubing attached to peristaltic pumps or similar devices controlled by a microprocessor programmed to meter the exact quantities of the materials in the correct sequence.

Detailed Description Text (108):
One example of such an automated process is depicted by a porous frit 13 in a glass or plastic vessel 15 shown in FIG. 3. The insoluble matrix 11 consists of a solid support such as cellulose, SEPHAROSE.TM. or CPG to which a nucleotide, nucleoside or polynucleotide is covalently linked at the 5'-position of the terminal nucleotide or to which an oligo- or poly-nucleotide or nucleoside having a terminal nucleoside with a free 3'-hydroxyl group is covalently attached via the 5'-hydroxyl group. The matrix II may itself be a covalent component of the porous frit 13 or it may be a separate

entity.

Detailed Description Text (110):
The stock containers 21, 23, 25 and 27 contain buffer solutions 51, 53, 55 and 57, respectively, having a concentration between about 10 and about 500 mM of sodium cacodylate (pH 7.0 at 25.degree. C.), between about 0.1 and about 1.0 mM of dithiothreitol. Each buffer solution also contains between about 0.10 and about 200 units per .mu.L of an enzyme (e.g. TdTase) suitable for phosphodiester bond formation. Buffer solution 51 in stock container 21 also contains between about 0.20 and about 200 .mu.M of deoxyadenosine 5'-triphosphate having a blocked 3'-hydroxyl group. Buffer solutions 53, 55 and 57 in stock containers 23, 25 and 27, respectively, contain equivalent concentrations of deoxycytosine 5'-triphosphate, deoxyguanosine 5'-triphosphate and thymidine 5'-triphosphate respectively, each of which also has blocked 3'-hydroxyl groups. Buffer solution 58 in stock container 28 contains an appropriate reagent for deblocking the blocked 3'-hydroxyl groups of the four nucleosides as described previously. Stock solution 59 in stock container 29 contains a suitable neutralization buffer at pH 7.0, such as 0.1M sodium cacodylate. Stock solution 30 in container 60 contains a suitable enzymatic solution or chemical reagent for releasing the final product from the solid support as described previously.

Detailed Description Text (114):
Finally, after the desire oligo- or polynucleotide is synthesized, cleavage of the object polynucleotide from the solid support occurs by the controlled addition of solution 60 which can be a restriction endonuclease solution or a solution to effect the chemical cleavage from the solid support (e.g., 1M piperidine) as described above. The microprocessor directs the distributor 71 and pump 93 to move the final product through tube 73 to be recovered for final workup.

Detailed Description Text (117):
The alternative strategy envisions the use of immobilized nucleotide triphosphates in order to separate the object nucleotide from non-reacting substrate polynucleotides at every cycle. The automated process using immobilized nucleotide is considerably different from the process involving an immobilized substrate polynucleotide. After the coupling reaction of the triphosphate and the polynucleotide, the eluate contains unreacted polynucleotides, reaction buffer, and TdTase enzyme. The object polynucleotide is attached to the solid support. In order to recycle the enzyme back to its reservoir, the contaminating polynucleotide is first removed by passing the solution through a column containing hydroxyl apatite, for example, or a similar polynucleotide adsorption medium through which the enzyme will pass. This column will have sufficient capacity to adsorb all of the anticipated contaminating polynucleotides produced by every cycle.

Detailed Description Text (119):
An automated process incorporating the immobilized nucleotide triphosphate alternative method of the present invention is depicted in FIG. 4. The process utilizes a nucleotide triphosphate immobilized to a solid support by, but not limited to, techniques describe previously, and compromising stock solutions 151, 153, 155, 157 in stock containers 121, 123, 125, 127. The stock solutions contain a tethered nucleotide, appropriate buffers and sufficient enzyme to effect the synthesis of the desired amount of predetermined sequence. The immobilization material has fluid dynamic properties allowing it to be moved through the various tubes as required. Substances which have these characteristics (e.g. gels and viscous suspensions) are familiar to one of ordinary skill in the art. The reaction vessel, 115, contains a reaction chamber, 111, and a stopcock, 113. Stopcock 113 has three positions A, B, C. Position A aligns a hole of sufficient diameter with the tubing so as to allow the various components of the synthesis to pass unimpeded. Position B aligns a porous frit to which is covalently attached oligonucleotides of deoxyadenosine (dA) approximately 20 bases in length. The quantity of oligo dA is sufficient to anneal the entire quantity of oligo dT, attached to the initiating substrate as described above. In position B, only solutes can pass through and no immobilization material (e.g. those contained in solutions 151, 153, 155, 157). Position C closes all flow. Reaction chamber 111 contains the initiating substrate in water, solution 161. As mentioned above the initiating substrate contains oligo dT which is .gtoreq.20 nucleotides in length. Stock containers 121, 123, 125, 127, 128 and 129 are connected to the reaction vessel 115 by way of peristaltic tubing or some similar material to effect transport

of the reagents contained in the stock containers. Additionally, vessel 115 is connected to tubing, 181, which contains a distributor, 171 which serves to divert the flow of solutes either to tubing 183 or tubing 185 or recycled back to stock containers after passing through adsorption media (e.g. hydroxylapatite) contained in 130, 132, 134 or 136 via tubes 187, 189, 191 or 193. Tubing 183 feeds back to vessel 115; tubing 185 feeds into a discard container. Solute movement through the tubing is facilitated by pumps, 131, 133, 135, 137, 138, 139, 163 (e.g. peristaltic pumps) or similar devices which will force fluids, gels or viscous suspensions through tubing to desired destinations.

Detailed Description Text (127):
It will be appreciated that for these separation techniques to be effective, the starting oligonucleotide or polynucleotide substrate should consist of at least approximately 20 nucleotides. The composition of the starting oligonucleotide or polynucleotide can be anything that will enable the subsequent purification steps as well as the ultimate cleavage of the object oligonucleotide or polynucleotide from the starting oligo- or polynucleotide. An example of a nucleotide modification that would enable final separation of starting oligonucleotide or polynucleotide from the object polynucleotide is biotinylation of the primary amines of dA, dC, or dG. Additionally, a starting oligonucleotide substrate containing 7-methyl guanosine at the 3' end will provide a cleavage site, as described previously, for ultimate recovery of the object polynucleotide.

Detailed Description Text (128):
Thus, it can be appreciated that, regardless of the equipment employed, the method of the present invention efficiently produces oligonucleotide or polynucleotides in high yield, with a significant reduction in the number of unreacted sequences per cycle. This greatly simplifies the ultimate isolation of the object nucleotide chain for further experimentation. Once isolated, the nucleotide chain may be "stitched" together with other polynucleotides and formed into double stranded DNA as described above or may be amplified by conventional means such as by polymerase chain reactions for use in recombinant DNA end use applications.

Detailed Description Text (131):
A kit for carrying out the instant synthesis may also contain initiating substrates that are attached to a solid support. The kit may contain a variety of initiating substrates attached to solid supports, so that the first nucleoside of a desired oligonucleotide can be selected by selecting the appropriate initiating substrate.

Detailed Description Text (195):
To demonstrate the synthesis of a desired DNA sequence directly onto a vector DNA by the TdTase catalyzed addition of the protected dNTPs, we performed sequential reactions on Pst 1-digested Puc 8 DNA in order to introduce a new restriction site into the vector. The sequence at the termini of the Pst1 Puc 8 DNA is: ##STR16## where the dotted lines indicate the annealed complementary strands of the vector. Sequential coupling and cleavage reaction were performed using the toluoyl esters of dNTPs as follows:

CLAIMS:

1. A nucleoside 5'-triphosphate having a removable blocking moiety protecting the 3' position which is selected from the group consisting of esters, ethers, carbonitriles, phosphates, carbonates, carbamates, borates, nitrates, sugars, phosphoramidates, phenylsulfanates, sulfates and sulfones, wherein said removable blocking moiety is linked to the 3' carbon of said nucleoside 5'-triphosphate.

2. A nucleoside 5'-triphosphate having a removable blocking moiety protecting the 3' position having the following formula: ##STR19## wherein R.sub.2 is triphosphate and wherein R.sub.1 is selected from the group consisting of phosphate, phosphoramidate and phosphoramide.

6. The nucleoside 5'-triphosphate of claim 2 wherein said removable blocking moiety is linked to a solid support.

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                ACID#)(10A) CHEMIC### (10A) CLEAV####

=> s l1 and (phosphothioate# or phosphoramidate#)
L2            0 L1 AND (PHOSPHOTHIOATE# OR PHOSPHORAMIDATE#)

=> s l1 and (end or termin####)
L3           21 L1 AND (END OR TERMIN####)

=> s l3 and (phosphrothioate# or phosphoramidate#)
L4            0 L3 AND (PHOSPHROTHIOATE# OR PHOSPHORAMIDATE#)

=> s l3 and immobiliz#####
L5            0 L3 AND IMMOBILIZ#####

=> dup rem l3
PROCESSING COMPLETED FOR L3
L6           10 DUP REM L3 (11 DUPLICATES REMOVED)

=> d l6 1-10 bib ab kwic

L6   ANSWER 1 OF 10  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
     DUPLICATE 1
AN   2000:418379  BIOSIS
DN   PREV200000418379
TI   Phosphorylation-induced signal propagation in the response regulator NtrC.
AU   Lee, Jonghui; Owens, Jeffrey T.; Hwang, Ingyu; Meares, Claude; Kustu,
     Sydney (1)
CS   (1) Department of Plant and Microbial Biology, University of California
     Berkeley, 111 Koshland Hall, No. 3102, Berkeley, CA, 94720-3102 USA
SO   Journal of Bacteriology, (September, 2000) Vol. 182, No. 18, pp.
     5188-5195. print.
     ISSN: 0021-9193.
DT   Article
LA   English
SL   English
AB   The bacterial enhancer-binding protein NtrC is a well-studied response
     regulator in a two-component regulatory system. The amino (N)-
     terminal receiver domain of NtrC modulates the function of its
     adjacent output domain, which activates transcription by the sigma54
     holoenzyme. When a specific aspartate residue in the receiver domain of
     NtrC is phosphorylated, the dimeric protein forms an oligomer
     that is capable of ATP hydrolysis and transcriptional activation. A
     chemical protein cleavage method was used to investigate
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signal propagation from the phosphorylated receiver domain of NtrC, which acts positively, to its central output domain. The iron chelate reagent Fe-BABE was conjugated onto unique cysteines introduced into the N-**terminal** domain of NtrC, and the conjugated proteins were subjected to Fe-dependent cleavage with or without prior phosphorylation. Phosphorylation-dependent cleavage, which requires proximity and an appropriate orientation of the peptide backbone to the tethered Fe-EDTA, was particularly prominent with conjugated NtrCD86C, in which the unique cysteine lies near the top of alpha-helix 4. Cleavage occurred outside the receiver domain itself and on the partner subunit of the derivatized monomer in an NtrC dimer. The results are commensurate with the hypothesis that alpha-helix 4 of the phosphorylated receiver domain of NtrC interacts with the beginning of the central domain for signal propagation. They imply that the phosphorylation-dependent interdomain and intermolecular interactions between the receiver domain of one subunit and the output domain of its partner subunit in an NtrC dimer precede-and may give rise to-the oligomerization needed for transcriptional activation.

AB The bacterial enhancer-binding protein NtrC is a well-studied response regulator in a two-component regulatory system. The amino (N)-**terminal** receiver domain of NtrC modulates the function of its adjacent output domain, which activates transcription by the sigma54 holoenzyme. When a specific aspartate residue in the receiver domain of NtrC is phosphorylated, the dimeric protein forms an **oligomer** that is capable of ATP hydrolysis and transcriptional activation. A **chemical** protein **cleavage** method was used to investigate signal propagation from the phosphorylated receiver domain of NtrC, which acts positively, to its central output domain. The iron chelate reagent Fe-BABE was conjugated onto unique cysteines introduced into the N-**terminal** domain of NtrC, and the conjugated proteins were subjected to Fe-dependent cleavage with or without prior phosphorylation. Phosphorylation-dependent cleavage, which. . .

L6 ANSWER 2 OF 10 MEDLINE on STN DUPLICATE 2
AN 92389315 MEDLINE
DN 92389315 PubMed ID: 1518042
TI Effect of sequence mutations on the higher order structure of the yeast 5 S rRNA.
AU Van Ryk D I; Nazar R N
CS Department of Molecular Biology and Genetics, University of Guelph, Ontario, Canada.
SO JOURNAL OF MOLECULAR BIOLOGY, (1992 Aug 20) 226 (4) 1027-35.
Journal code: 2985088R. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199210
ED Entered STN: 19921023
Last Updated on STN: 19921023
Entered Medline: 19921006

AB Mutant yeast ribosomal 5 S RNAs were **probed** by enzymatic **cleavage** and **chemical** reactivity to define further the higher order structure. Mutations that destabilized helix IV resulted in an altered tertiary structure in which a reduced reactivity to ethylnitrosourea at U90 and G91 could be correlated with greater enzymatic and Fe(II)-EDTA cleavages in helices II and V. The results provide direct evidence for, and a further definition of, a structural juxtaposition between helix II and the **end** of helix IV and indicate that, in contrast to earlier suggestions, the remaining tertiary structure is sufficiently stable to prevent "pseudoknot-like" interactions between helices III and IV. The data are fully consistent with the "lollipop" model of the tertiary structure.

AB Mutant yeast ribosomal 5 S RNAs were **probed** by enzymatic **cleavage** and **chemical** reactivity to define further the

higher order structure. Mutations that destabilized helix IV resulted in an altered tertiary structure in. . . V. The results provide direct evidence for, and a further definition of, a structural juxtaposition between helix II and the **end** of helix IV and indicate that, in contrast to earlier suggestions, the remaining tertiary structure is sufficiently stable to prevent. . .

L6 ANSWER 3 OF 10 MEDLINE on STN DUPLICATE 3
AN 92089081 MEDLINE
DN 92089081 PubMed ID: 1721536
TI Drug binding to a DNA BZ molecule: analysis by chemical footprinting.
AU Guo Q; Lu M; Shahrestanifar M; Sheardy R D; Kallenbach N R
CS Department of Chemistry, New York University, New York 10003.
NC CA-24101 (NCI)
SO BIOCHEMISTRY, (1991 Dec 24) 30 (51) 11735-41.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199201
ED Entered STN: 19920216
Last Updated on STN: 19960129
Entered Medline: 19920124
AB The polymorphism in a DNA 16-mer (designated BZ-II) has been investigated by means of circular dichroism (CD) spectroscopy and chemical footprinting. CD spectra indicate that, in low salt, the oligomer is fully right-handed whereas, in high salt, it possesses a B-Z conformational junction: half of the duplex is right-handed while the other half is left-handed. Treatment of BZ-II with diethyl pyrocarbonate (DEPC) confirms the existence of a left-handed segment of the duplex in high salt: enhanced DEPC scission occurs at the G residues in the alternating CG sequence. The scission patterns of the upper and lower strands in BZ-II by the reactive chemical probe MPE.Fe(II), and the antitumor antibiotics dynemicin and Fe-(II).bleomycin, are different under low salt conditions. The 3'-**terminal** region of both upper and lower strands and the middle region of the upper strand of BZ-II are preferential cleavage sites in low salt. This result suggests that the methylated cytosines or the alternating CG domain in the molecule perturbs the DNA structure. Under high salt conditions, the reactivity of the Z-DNA segment of BZ-II for MPE.Fe(II) and Fe(II).bleomycin is dramatically enhanced, while it is reduced in the case of dynemicin. Excess propidium (PI) eliminates preferential **cleavage** by each of these **chemical probes** in high salt conditions. This is due in part to conversion of the BZ-DNA molecule into B-DNA, as is seen by a DEPC modification experiment.(ABSTRACT TRUNCATED AT 250 WORDS)
AB . . . by the reactive chemical probe MPE.Fe(II), and the antitumor antibiotics dynemicin and Fe-(II).bleomycin, are different under low salt conditions. The 3'-**terminal** region of both upper and lower strands and the middle region of the upper strand of BZ-II are preferential cleavage. . . MPE.Fe(II) and Fe(II).bleomycin is dramatically enhanced, while it is reduced in the case of dynemicin. Excess propidium (PI) eliminates preferential **cleavage** by each of these **chemical probes** in high salt conditions. This is due in part to conversion of the BZ-DNA molecule into B-DNA, as is seen. . .

L6 ANSWER 4 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 4
AN 1991:271917 BIOSIS
DN BA92:4532
TI ISOLATION AND CHARACTERIZATION OF A COMPLEMENTARY DNA CLONE ENCODING AN 18-KDA HYDROPHOBIC PHOTOSYSTEM I SUBUNIT PSI-L FROM BARLEY HORDEUM-VULGARE L.

AU OKKELS J S; SCHELLER H V; SVENDSEN I; MOLLER B L
CS PLANT BIOCHEM. LAB., DEP. PLANT BIOL., ROYAL VETERINARY AGRIC. UNIV., 40 THORVALDSENSVEJ, DK-1871 FREDERIKSBERG C, COPENHAGEN, DENMARK.
SO J BIOL CHEM, (1991) 266 (11), 6767-6773.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB Photosystem I in barley contains a polypeptide with an apparent molecular mass of 14 kDa. The polypeptide is N-**terminally** blocked to amino acid sequencing, but partial amino acid sequences have been determined from three fragments obtained by **chemical** and enzymatic **cleavage**. Using an oligonucleotide **probe** specifying this amino acid sequence, a full length cDNA clone was isolated. The deduced amino acid sequence does not correspond to any previously identified photosystem I subunit. We designate the novel photosystem I subunit PSI-L and the corresponding nuclear gene PsaL. The cDNA clone encodes a precursor polypeptide of 209 amino acid residues with a deduced molecular mass of 22,210 Da. The precursor has a transit peptide typical of proteins imported into chloroplasts. Based on a putative maturation site, the deduced molecular mass of the mature protein is 18 kDa. The PSI-L polypeptide is hydrophobic and predicted to have at least two membrane-spanning .alpha.-helices. Northern blot analysis shows the expression of the PsaL gene is light-induced similar to other of the barley photosystem I genes. Southern blot analysis indicates that PsaL is a single copy gene. Partial amino acid sequences of an N-**terminally** blocked 9-kDa polypeptide show high sequence similarity to the PSI-G polypeptide of spinach and Chlamydomonas reinhardtii. The gene product of PsaG in spinach has previously been assigned as subunit V (Steppuhn, J., Hermans, J., Nechushtai, R., Ljungberg, U., Thummler, F., Lottspeich, F., and Herrmann, R. G. (1988) FEBS Lett. 237, 218-224). The present study suggests that PSI-L is equivalent to subunit V and that PSI-G is a subunit migrating closely to PSI-H (subunit VI) and PSI-C (subunit VII).
AB Photosystem I in barley contains a polypeptide with an apparent molecular mass of 14 kDa. The polypeptide is N-**terminally** blocked to amino acid sequencing, but partial amino acid sequences have been determined from three fragments obtained by **chemical** and enzymatic **cleavage**. Using an oligonucleotide **probe** specifying this amino acid sequence, a full length cDNA clone was isolated. The deduced amino acid sequence does not correspond. . . photosystem I genes. Southern blot analysis indicates that PsaL is a single copy gene. Partial amino acid sequences of an N-**terminally** blocked 9-kDa polypeptide show high sequence similarity to the PSI-G polypeptide of spinach and Chlamydomonas reinhardtii. The gene product of. . .

L6 ANSWER 5 OF 10 MEDLINE on STN DUPLICATE 5
AN 91088284 MEDLINE
DN 91088284 PubMed ID: 2263464
TI Structural analysis of the 5' domain of the HeLa 18S ribosomal RNA by chemical and enzymatic probing.
AU Mandiyan V; Boublik M
CS Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.
SO NUCLEIC ACIDS RESEARCH, (1990 Dec 11) 18 (23) 7055-62.
Journal code: 0411011. ISSN: 0305-1048.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199102
ED Entered STN: 19910322
Last Updated on STN: 19970203
Entered Medline: 19910201
AB The secondary structure of HeLa 18S rRNA was investigated by a combination

of chemical and enzymatic probing techniques. Using four chemical reagents (DMS*, kethoxal, DEPC and CMCT) which react specifically with unpaired bases and two nucleases (RNase T1 and cobra venom nuclease) which cleave the ribopolynucleotides at unpaired guanines and helical segments, we have analyzed the secondary structure of the 5' domain of 18S rRNA isolated from HeLa 40S ribosomal subunits. The sites at which **chemical** modifications and nuclease **cleavages** occurred were identified by **primer** extension using synthetic deoxyoligonucleotides and reverse transcriptase. These studies led to the deduction of an intra-RNA pairing pattern from the available secondary structure models based on comparative sequence analysis. Apart from the general canonical pairing we have identified noncanonical U-U, G-A, A-G, A-C, C-A and G-G pairing in HeLa 18S rRNA. The differential reactivity of bases to chemical reagents has enabled us to predict the possible configuration of these bases in some of the noncanonical pairing. The absence of chemical reactivities and cobra venom nuclease sensitivity in the **terminal** loops of helices 6 and 12 indicate a tertiary interaction unique to HeLa 18S rRNA. We have confirmed the existence of the complex tertiary folding recently proposed (Gutell and Woese 1990 Proc. Natl. Acad. Sci. 87, 663-667) for the universally conserved helix 19 in HeLa 18S rRNA. The complementarity of chemical modifications and enzymatic cleavages provided experimental evidence for the proposal of a model structure for the 655 nucleotides of the 5' domain of HeLa 18S rRNA.

AB . . . the secondary structure of the 5' domain of 18S rRNA isolated from HeLa 40S ribosomal subunits. The sites at which **chemical** modifications and nuclease **cleavages** occurred were identified by **primer** extension using synthetic deoxyoligonucleotides and reverse transcriptase. These studies led to the deduction of an intra-RNA pairing pattern from the. . . these bases in some of the noncanonical pairing. The absence of chemical reactivities and cobra venom nuclease sensitivity in the **terminal** loops of helices 6 and 12 indicate a tertiary interaction unique to HeLa 18S rRNA. We have confirmed the existence. . .

L6 ANSWER 6 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1990:360993 BIOSIS
DN BA90:57572
TI LOCALIZATION OF THE MEMBRANE-ASSOCIATED REGION OF VESICULAR STOMATITIS VIRUS M PROTEIN AT THE AMINO **TERMINUS** USING THE HYDROPHOBIC PHOTOREACTIVE PROBE IODINE-125 TID.
AU LENARD J; VANDEROEF R
CS DEP. PHYSIOL. AND BIOPHYSICS, ROBERT WOOD JOHNSON MED. SCH., UNIV. MED. AND DENTISTRY N.J., 675 HOES LANE, PISCATAWAY, N.J. 08854-5635.
SO J VIROL, (1990) 64 (7), 3486-3491.
CODEN: JOVIAM. ISSN: 0022-538X.
FS BA; OLD
LA English
AB The membrane-reactive, photoactivatable probe 125I-TID {3-(trifluoromethyl)-3-(m-[125I]iodophenyl)-3H-diazirine} was found to label the M protein of vesicular stomatitis virus about 40% as much as G protein in intact virions, in agreement with labeling studies with other **probes**. By analyzing limited tryptic digestion and specific **chemical cleavage** products, the label was essentially entirely localized within the first 19, and probably within the first 5 to 10, amino acid residues at the N **terminus**, identifying this short amphipathic segment as the likely site of interaction of M protein with the viral bilayer.
TI LOCALIZATION OF THE MEMBRANE-ASSOCIATED REGION OF VESICULAR STOMATITIS VIRUS M PROTEIN AT THE AMINO **TERMINUS** USING THE HYDROPHOBIC PHOTOREACTIVE PROBE IODINE-125 TID.
AB. . . vesicular stomatitis virus about 40% as much as G protein in intact virions, in agreement with labeling studies with other **probes**. By analyzing limited tryptic digestion and specific **chemical**

cleavage products, the label was essentially entirely localized within the first 19, and probably within the first 5 to 10, amino acid residues at the N **terminus**, identifying this short amphipathic segment as the likely site of interaction of M protein with the viral bilayer.

L6 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1988:607645 CAPLUS
DN 109:207645
TI Double strand cleavage of genomic DNA at a single site by triple helix formation
AU Strobel, Scott A.; Moser, Heinz E.; Dervan, Peter B.
CS Arnold and Mabel Beckman Lab. Chem. Synth., California Inst. Technol., Pasadena, CA, 91125, USA
SO Journal of the American Chemical Society (1988), 110(23), 7927-9
CODEN: JACSAT; ISSN: 0002-7863
DT Journal
LA English
AB An oligonucleotide-EDTA-Fe probe (0.8 .mu.M) equipped with thymidine-EDTA (T*) at the 5' **end**, 5'-T*T3CT6CT4CT-3', causes double-strand cleavage at a single homopurine site 18 base pairs in size (5'-A4GA6GA4GA-3') within 48,502 base pairs of bacteriophage .lambda. DNA (1 .mu.M in base pairs). The double-strand cleavage efficiency is 25% (100 mM NaCl, 25 mM tris acetate (pH 7.0), 1 mM spermine, 24.degree.). No secondary cleavage sites (at partially homologous sequences) were detected under these reaction conditions. The oligonucleotide-EDTA-Fe-mediated site-specific double-strand cleavage of DNA also can be carried out in a low-melting-point agarose matrix. This work has implications for isolating genes from large chromosomes and mapping large genomes.
AB An oligonucleotide-EDTA-Fe probe (0.8 .mu.M) equipped with thymidine-EDTA (T*) at the 5' **end**, 5'-T*T3CT6CT4CT-3', causes double-strand cleavage at a single homopurine site 18 base pairs in size (5'-A4GA6GA4GA-3') within 48,502 base pairs of bacteriophage .lambda. DNA (1 .mu.M in base pairs). The double-strand cleavage efficiency is 25% (100 mM NaCl, 25 mM tris acetate (pH 7.0), 1 mM spermine, 24.degree.). No secondary cleavage sites (at partially homologous sequences) were detected under these reaction conditions. The oligonucleotide-EDTA-Fe-mediated site-specific double-strand cleavage of DNA also can be carried out in a low-melting-point agarose matrix. This work has implications for isolating genes from large chromosomes and mapping large genomes.
IT Chains, **chemical**
(**cleavages** of, of double-stranded DNA with oligonucleotide-EDTA-iron **probe**)

L6 ANSWER 8 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1988:469873 CAPLUS
DN 109:69873
TI Sequence-targeted cleavage of nucleic acids by oligo-[.alpha.]thymidylate-phenanthroline conjugates: parallel and antiparallel double helices are formed with DNA and RNA, respectively
AU Sun, Jian Sheng; Francois, Jean Christophe; Lavery, Richard; Saison-Behmoaras, Tula; Montenay-Garestier, Therese; Nguyen Thanh Thuong; Helene, Claude
CS Lab. Biophys., Mus. Natl. Hist. Nat., Paris, 75005, Fr.
SO Biochemistry (1988), 27(16), 6039-45
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB Oligodeoxynucleotides can be synthesized by using the .alpha. anomers of nucleoside units. Oligo-.alpha.-deoxynucleotides are resistant to nucleases and could be used to regulate gene expression in vivo. Theor. calcns. were done to det. the conformational energy of an oligomeric .alpha.-.beta. duplex (dA)5.cntdot.(dT)5 where the adenosine strand contains natural .beta.-deoxyribunucleotides and the thymidine strand

contains synthetic .alpha.-deoxyribonucleotides. These calcns. predict that in the more stable B-like conformation, the 2 strands of the double helix should run parallel to each other whereas in the more stable A-like conformation, the 2 strands should adopt an antiparallel orientation. To test these predictions, 1,10-phenanthroline was covalently attached to the 5'-**end** of an .alpha.-octathymidylate. In the presence of Cu ions and a reducing agent (.beta.-mercaptopropionic acid), the (phenanthroline2-Cu complex generates OH.bul. radicals that cleave phosphodiester bonds in the complementary sequence at which the .alpha.-octathymidylate is bound. By using a 27mer oligo-.beta.-deoxynucleotide contg. an octadeoxyadenylate sequence as a target for the phenanthroline-substituted .alpha.-(dT)8 cleavage was obsd. on the 5'-side of the (dA)8 sequence, demonstrating that the .alpha.-.beta. DNA-DNA hybrid formed a double helix with parallel orientation of the 2 strands. The same result was obtained when .alpha.-(dT)8 was bound to .beta.-(dA)n with n = 8 or 10. When a .beta.-oligoriboadenylate was used as a target, cleavage occurred exclusively on the 3'-side of the (rA)8 or (rA)10 sequence, indicating that the .alpha.-.beta. DNA-RNA hybrid formed a double helix with an antiparallel orientation of the 2 strands. When a phenanthroline-substituted .beta.-octathymidylate was used instead of the .alpha.-octathymidylate, an antiparallel double helix was formed independently of whether the target .beta. sequence was a DNA or an RNA.

AB Oligodeoxynucleotides can be synthesized by using the .alpha. anomers of nucleoside units. Oligo-.alpha.-deoxynucleotides are resistant to nucleases and could be used to regulate gene expression in vivo. Theor. calcns. were done to det. the conformational energy of an oligomeric .alpha.-.beta. duplex (dA)5.cntdot.(dT)5 where the adenosine strand contains natural .beta.-deoxyribunucleotides and the thymidine strand contains synthetic .alpha.-deoxyribonucleotides. These calcns. predict that in the more stable B-like conformation, the 2 strands of the double helix should run parallel to each other whereas in the more stable A-like conformation, the 2 strands should adopt an antiparallel orientation. To test these predictions, 1,10-phenanthroline was covalently attached to the 5'-**end** of an .alpha.-octathymidylate. In the presence of Cu ions and a reducing agent (.beta.-mercaptopropionic acid), the (phenanthroline2-Cu complex generates OH.bul. radicals that cleave phosphodiester bonds in the complementary sequence at which the .alpha.-octathymidylate is bound. By using a 27mer oligo-.beta.-deoxynucleotide contg. an octadeoxyadenylate sequence as a target for the phenanthroline-substituted .alpha.-(dT)8 cleavage was obsd. on the 5'-side of the (dA)8 sequence, demonstrating that the .alpha.-.beta. DNA-DNA hybrid formed a double helix with parallel orientation of the 2 strands. The same result was obtained when .alpha.-(dT)8 was bound to .beta.-(dA)n with n = 8 or 10. When a .beta.-oligoriboadenylate was used as a target, cleavage occurred exclusively on the 3'-side of the (rA)8 or (rA)10 sequence, indicating that the .alpha.-.beta. DNA-RNA hybrid formed a double helix with an antiparallel orientation of the 2 strands. When a phenanthroline-substituted .beta.-octathymidylate was used instead of the .alpha.-octathymidylate, an antiparallel double helix was formed independently of whether the target .beta. sequence was a DNA or an RNA.

IT Chains, **chemical**
(orientation of, in double-helical DNA contg. .alpha.-anomeric chain, **nucleic acid** sequence-specific **cleavage** by octathymidylate anomer phenanthroline-substituted derivs. in relation to)

L6 ANSWER 9 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1985:467847 CAPLUS
DN 103:67847
TI Site-specific cleavage of tobacco mosaic virus RNA: a study of factors influencing the cleavage
AU Saleem, M.; Pelcher, L. E.
CS Plant Biotechnol. Inst., Natl. Res. Counc. Canada, Saskatoon, SK, S7N 0W9, Can.

SO Canadian Journal of Biochemistry and Cell Biology (1985), 63(5), 382-6
CODEN: CJBBDU; ISSN: 0714-7511
DT Journal
LA English
AB DNA oligomer directed RNase H methodol. was applied to specifically cleave tobacco mosaic virus (TMV) RNA. A synthetic DNA oligomer P(dT8)dCdC, complementary to a region from nucleotide 5545 to nucleotide 5554 at the 3' **end** of TMV RNA, was used to cleave the RNA at the site of polynucleotides complementary to the DNA oligomer. Factors such as secondary structure of the RNA, concns. of DNA oligomer, RNase H, and Mg2+ in the reaction mixt., and time of incubation were optimized for the RNase H cleavage of TMV RNA-DNA oligomer complex. Denaturation of TMV RNA with 50% DMSO at 50.degree. is essential for the site-specific cleavage.
AB DNA oligomer directed RNase H methodol. was applied to specifically cleave tobacco mosaic virus (TMV) RNA. A synthetic DNA oligomer P(dT8)dCdC, complementary to a region from nucleotide 5545 to nucleotide 5554 at the 3' **end** of TMV RNA, was used to cleave the RNA at the site of polynucleotides complementary to the DNA oligomer. Factors such as secondary structure of the RNA, concns. of DNA oligomer, RNase H, and Mg2+ in the reaction mixt., and time of incubation were optimized for the RNase H cleavage of TMV RNA-DNA oligomer complex. Denaturation of TMV RNA with 50% DMSO at 50.degree. is essential for the site-specific cleavage.
IT Chains, **chemical**
(**cleavage** of, site-specific, of RNA, DNA **oligomer** and RNase H in)

L6 ANSWER 10 OF 10 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7
AN 1976:205243 BIOSIS
DN BA62:35243
TI **CHEMICAL PROBES** OF EXTENDED BIOLOGICAL STRUCTURES
SYNTHESIS AND PROPERTIES OF THE **CLEAVABLE** PROTEIN CROSS LINKING REAGENT SULFUR-35 DI THIO BIS SUCCINIMIDYL PROPIONATE.
AU LOMANT A J; FAIRBANKS G
SO J MOL BIOL, (1976) 104 (1), 243-261.
CODEN: JMOBAK. ISSN: 0022-2836.
FS BA; OLD
LA Unavailable
AB The synthesis and properties of a new cleavable protein cross-linking reagent, [35S]dithiobis(succinimidyl propionate), are detailed. Free primary and secondary aliphatic amino groups are quantitatively acylated by the reagent in either organic or aqueous media within 2 min at 23.degree. C. The half-time for hydrolysis of the active ester **termini** in buffer at pH 7 is 4-5 h, so that protein cross-linkage can be optimized by application of low concentrations of reagent. Accessible amino groups of bovine Hb are acylated with extreme rapidity of 0.degree. C in pH 7 buffer when [35S]dithiobis(succinimidyl propionate) is applied in 0.4 to 9-fold molar excess. Submicrogram quantities of the cross-linked Hb subunits which result are detectable by monitoring the 35S distribution in sodium dodecyl sulfate-polyacrylamide gels. In addition to amine acylation, 2 of the 6 thiol groups in Hb, tentatively located at cysteine 93 of the .beta. chains, are reversibly modified at 0.degree. C by mercaptan-disulfide interchange with the reagent or its bis amide analogs. This equilibrium-controlled, pH-dependent reaction occurs at a slower rate than acylation, and is blocked by short preincubation of the protein with N-ethylmaleimide or by addition of 3,3'-dithiodipropionamide (or other disulfides) to the reaction mixture. Disulfides introduced into Hb by acylation and interchange are quantitatively cleaved by reduction for 30 min at 37.degree. C with 10 mM-dithioerythritol buffered at pH 8.5. The properties of high reactivity under mild conditions, long solution half-life, and the radioactive label make (35S]dithiobis(succinimidyl propionate) a particularly useful and versatile probe of extended structures in a variety of biological systems.
TI **CHEMICAL PROBES** OF EXTENDED BIOLOGICAL STRUCTURES

SYNTHESIS AND PROPERTIES OF THE **CLEAVABLE** PROTEIN CROSS LINKING REAGENT SULFUR-35 DI THIO BIS SUCCINIMIDYL PROPIONATE.

AB. . . in either organic or aqueous media within 2 min at 23.degree. C. The half-time for hydrolysis of the active ester **termini** in buffer at pH 7 is 4-5 h, so that protein cross-linkage can be optimized by application of low concentrations. . .

=>